## REMARKS

The present application is directed to a method for detecting the presence of a target nucleic acid sequence by amplifying a target nucleic acid, wherein the target contains a purine rich region or wherein a purine-rich region is introduced into the target during amplification, adding a peptide nucleic acid (PNA), and detecting the presence of triplex structures. Upon entry of the present amendment, Claims 1-2, 5-6, 8-12, 14, 16, and 18-24 will be pending in this application.

Clarification of Office Action

Applicants respectfully request clarification of the Office Action mailed December 11, 2003. It appears from the Office Action that the Examiner did not receive the Second Preliminary Amendment filed August 6, 2003. In the Office Action mailed December 11, 2003, the Examiner acknowledges the Preliminary Amendment filed with the Request for Continued Examination (RCE) on June 23, 2003, but does not mention the Second Preliminary Amendment filed August 6, 2003. A copy of the Second Preliminary Amendment is enclosed. Applicants respectfully request entry of the amendments to the claims presented in the Second Preliminary Amendment and assume, for the purposes of this Response, that the amendments will be entered.

In addition, the cover sheet of the Office Action mailed December 11, 2003, omits Claims 14 and 15. Both claims were pending upon entry of the first Preliminary Amendment. Subsequently, Claim 14 was amended and Claim 15 was canceled in the Second Preliminary Amendment filed August 6, 2003. However, even though not listed on the cover sheet, it appears that Claim 14, in unamended form, was considered by the Examiner in the Office Action mailed December 11, 2003, because it was rejected as obvious in the body of the Office Action.

Applicants respectfully request confirmation that the Amendment submitted in the Second Preliminary Amendment has been entered and that the Claims Listing provided herein is correct. Applicants also respectfully request that, even if the claims are not yet found to be allowable, the Examiner issue an Office Action that is non-final.

Rejections Under 35 U.S.C. §103

In the Office Action mailed December 11, 2003, the Examiner rejected Claims 1-2, 5-6, 8, 12, 14, 22 and 24 under 35 U.S.C. §103(a) as unpatentable over Vary (WO 92/11390), in view of Ecker *et al.* (US 5,641,625) in further view of Gildea *et al.* (WO 99/21881). Applicants respectfully traverse this rejection.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine the teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP 2143. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The level of skill in the art cannot be relied upon to provide the suggestion to combine references. *Al-site Corp. v. VSI Int'l Inc.*, 174 F.3d 1308, 50 USPQ2d 1161 (Fed. Cir. 1999).

Vary teach use of a nucleotide probe for the detection of a target nucleic acid sequence by forming a triple helix. As conceded by the Examiner, Vary fails to teach the use of PNAs to detect a target sequence. Applicants submit that Vary also fails to provide any suggestion that PNAs would be useful as probes in his detection method.

Ecker *et al.* teach that PNA probes bind with high stability and specificity to double-stranded DNA to form triple helices. Ecker *et al.* further teach that PNAs can be used to modulate gene expression, but can be used in diagnostics **only if** the target nucleic acid is single stranded (see col. 4, lines 63-67). Therefore, Ecker *et al.* fail to teach contacting a target sample with PNAs during amplification and fail to suggest that measurement of a triple helix can be an indicator of the presence of the target sequence.

The present application claims priority to PCT /GB99/02317, filed July 19, 1999, which claims priority to U.K. Patent Application No. 9815933.8, filed July 23, 1998. The Gildea *et al.* PCT application cited by the Examiner was published on May 6, 1999, and therefore is not a valid prior art reference under 35 U.S.C. §102(b). However, applicants have discovered related U.S. Patent No. 6,485,901 to Gildea *et al.*, a copy of which is enclosed. The Gildea *et al.* '901 patent claims priority to U.S. provisional patent application 60/063,283, which asserts a filing date of October 27, 1997. A copy of the provisional patent application is also enclosed for the Examiner's convenience. Applicants wish to note that portions of the issued Gildea *et al.* '901 patent are not entitled to the asserted priority date because those portions were not included in the provisional patent application. In particular, Example 19 was added to the Gildea *et al.* '901 patent and is not entitled to the priority date.

The Gildea *et al.* '901 patent teaches the use of a labeled PNA molecule (referred to as a "Linear Beacon") as a hybridization probe to detect the presence of a target sequence using Fluorescence Resonance Energy Transfer (FRET). The PNA molecule has a nucleobase sequence complementary to the target sequence. The nucleobase sequence is flanked by a donor moiety, such as a flurorphore, and an acceptor moiety, such as a quencher. In the absence of target, energy is transferred between the donor and acceptor moieties and donor signal is inhibited. In the presence of target, the PNA molecule hybridizes to the target and signal is detectable.

The Examiner asserts that one skilled in the art would have been motivated to use the PNA probes of Ecker *et al.* in the detection method taught by Vary to achieve applicants' claimed detection methods.

Independent Claims 1 and 6 of the present application specify that a sample is contacted with a PNA molecule during the amplification step. Claims 2, 5, 8, 12, 14, 22 and 24 depend from Claim 1 or 6. As explained in the Preliminary Amendment filed June 23, 2003, Ecker *et al.* teach that PNA probes bind with great stability. High stability binding such as described by Ecker *et al.* is similarly described in the Frank-Kamenetskii *et al.* reference (WO 97/14793) also cited by the Examiner. Frank-Kamenetskii *et al.* teach the use of PNA sequences as nucleic acid "clamps" for various uses including the inhibition of background signals (see page 17, lines 14-27 of Frank-Kamenetskii *et al.*). Frank-Kamenetskii *et al.* state that, once a PNA probe has bound to a sequence, it "inhibits further rounds of PCR amplification." Accordingly, one of ordinary skill in the art reading the Frank-Kamenetskii *et al.* paper would **avoid** the use of PNA probes during an amplification reaction because one would expect that the stability of the triplex would **significantly inhibit** amplification. Therefore, Ecker *et al.* (and Frank-Kamenetskii *et al.*) actually **teach away** from the claimed detection method.

The Examiner points to page 5, lines 23-32 and page 3, lines 12-15 of the Gildea *et al.* PCT application for the proposition that Gildea *et al.* teach PNA probes and detection "real time" during PCR. This is believed to be a misreading of this part of the specification. Applicants respectfully submit that both of the statements referenced by the Examiner are made by Gildea *et al.* in the "Background Art" section of the application and fail describe PNA detection methods at all.

On page 3, lines 12-15, Gildea *et al.* describe the nucleic acid probe constructs of Tyagi *et al.* (WO95/13399). The "assays of the invention" that Gildea *et al.* are describing on this page are the **nucleic acid** (Molecular Beacon) assays of Tyagi *et al.*, **not** PNA assays. Gildea *et al.* are merely describing Tyagi *et al.* as evidenced by the fact that Gildea *et al.* provide the relevant page and lines of the Tyagi *et al.* reference. (See page 3, line 14 of the Gildea *et al.* PCT application within the parentheses.) As Gildea *et al.* state clearly at the end of the same paragraph, the assays of Tyagi *et al.* are **not** PNA assays. "Neither Tyagi *et al.* nor Tyagi2 *et al.* disclose, suggest or teach anything about PNA." (Gildea *et al.*, page 3, lines 18-19.)

On page 5, lines 23-32, Gildea *et al.* merely define the term "PNA" and state that it is "neither a peptide, a nucleic acid, nor is it even an acid. Peptide Nucleic Acid (PNA) is a non-naturally occurring polyamide (pseudopeptide) which can hybridize to nucleic acid (DNA and RNA) with sequence specificity." In this cited section, Gildea *et al.* also provide information on how PNAs can be synthesized or purchased.

Therefore, applicants respectfully submit that Gildea *et al.* fail to teach the use of PNA probes for real time detection during PCR. In addition, Gildea *et al.* fail to disclose the formation of triplex molecules or the detection of triplex structures as a means for detecting a target sequence in a sample. Also, in relation to Claim 1 of the present application, Gildea *et al.* fail to disclose the introduction of a purine-rich region during amplification. Furthermore, Gildea *et al.* discourage the substitution of PNA probes for nucleic acid probes by emphasizing that PNA probes are not the equivalent of nucleic acid probes in both structure or function. (page 6, lines 9-13 and page 7, lines 13-24) "Consequently, the unique biological, structural, and physico-chemical properties of PNA requires that experimentation be performed to thereby examine whether PNAs are suitable in applications where nucleic acid probes are commonly utilized." (page 7, lines 22-24) This statement clearly admits that one of skill in the art would not be assured a reasonable expectation of success without experimentation. Therefore, taken as a whole, Gildea *et al.* actually teach away from combining the PNAs of Ecker *et al.* with the method disclosed by Vary and, even if the references were combined, there would be no reasonable expectation of success.

In the Office Action mailed December 11, 2003, the Examiner rejected Claims 9-11, 16, 18-21 and 23 under 35 U.S.C. § 103(a) as unpatentable over Vary (WO 92/11390), in view of

Ecker *et al.* (US 5,641,625) and Gildea *et al.* (WO 99/21881) in further view of Wang *et* al. (*J Am Chem Soc* 118: 7667-7670). Applicants respectfully traverse this rejection.

Vary, Ecker *et al.* and Gildea *et al.* have been discussed above. Wang discloses a biosensor attached to a PNA probe for detection of a nucleic acid system. Wang fails to disclose amplifying a target nucleic acid, introducing or utilizing a purine-rich region during amplification, or detecting the presence of triplex structures.

As discussed above, the Gildea *et al.* teach away from combining Vary and Ecker *et al.* to arrive at the claimed method with a reasonable expectation of success. Wang fails to make up for these insufficiencies. Absent teachings of the present specification, one of skill in the art would not have obtained sufficient motivation to combine these references to arrive at the claimed subject matter with a reasonable expectation of success.

In the Office Action mailed December 11, 2003, the Examiner rejected Claims 1, 2, 5, 6, and 8 under 35 U.S.C. § 103(a) as unpatentable over Vary (WO 92/11390), in view of Frank-Kamenetskii *et al.* (WO 97/14793) in further view of Gildea *et al.* (WO 99/21881). Applicants respectfully traverse this rejection.

Vary and Gildea *et al.* have been discussed above. Frank-Kamenetskii *et al.* disclose using bis-PNAs that are capable of binding to double stranded DNA in order to block gene expression. Bis-PNAs are used to protect restriction sites during methylation of the double stranded DNA. Bis-PNAs are used to prevent aberrant amplification of non-target sequences during PCR. Frank-Kamenetskii *et al.* fail to disclose using bis-PNAs to detect a target sequence and in fact do not disclose target-specific PNAs to detect a double stranded DNA sample at all. All embodiments disclosed in Frank-Kamenetskii *et al.* define functional and therapeutic uses of bis-PNAs.

Gildea *et al.* teach away from using the method of Vary for using PNAs to detect a target sequence by detecting the presence of triple helix structures. Frank-Kamenetskii *et al.* fails to overcome this deficiency. Absent the teachings of the specification, one of ordinary skill in the art would not possess the suggestion, motivation nor reason to combine the cited references to arrive at the claimed method with a reasonable expectation of success.

## CONCLUSION

Applicants respectfully submit that the claims are non-obvious in view of the cited references and are in condition for allowance. A Notice of Allowance is respectfully solicited. If there remain any additional issues to be addressed, the Examiner is invited to contact the undersigned attorney at 404-745-2473.

Respectfully submitted,

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